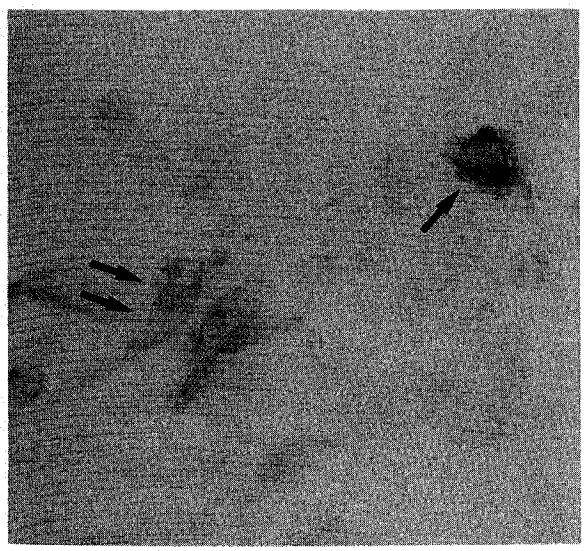
LETTERS to the EDITOR

HTLV-I transmission from mother to fetus via placenta

SIR,—The main route of mother-to-child transmission of human T-lymphotropic virus type I (HTLV-I) is thought to be by breastfeeding.[1] Thus some clinicians insist that such transmission can be prevented if HTLV-I-seropositive mothers do not breastfeed. Epidemiological data have now demonstrated several cases of HTLV-I infection among children who had not been breastfed.[2] There is also a report that HTLV-I proviral genome was detectable in cord-blood lymphocytes from HTLV-I carrier mothers,[3] suggesting that HTLV-I had infected the fetus in utero. We have used immunocytochemistry and polymerase chain reaction (PCR) to examine whether placentas from HTLV-I carrier mothers were infected by HTLV-I.

Placental villi were obtained from 12 pregnant women at term: 9 from HTLV-I-seropositive women and 3 from HTLV-I seronegative. Monolayer placental villous cells were obtained.[4] After 3 passages, cells on the coverslips reacted with GIN-14, a monoclonal antibody against HTLV-I gag protein (p19).

2 of the 9 specimens from HTLV-I-seropositive women stained positive against GIN-14. Double immunostaining revealed that these GIN-14-reactive cells were also reactive with PKK1, a monoclonal antibody to cytokeratin of epithelial cells (figure). This result shows that the GIN-14-reactive cells on the coverslips were placental villous cells and not lymphocytes, because lymphocytes do not react with PKK1.



Double immunostaining of cultured placental villous cells with GIN-14 and PKK-1.

Double arrows = double-staining of cell with GIN-14 (brown) and PKK-1 (blue-grey). Single arrow = cell reactive with PKK-1 only.

Using nested double PCR[5] with HTLV-I pX oligonucleotide primers,[6] we found that GIN-14-reactive placentas were positive for HTLV-I proviral DNA. 3 samples of placental villous cells from the HTLV-I-seronegative women were negative for HTLV-I antigen and proviral DNA.

Thus we found that 22% of placentas from HTLV-I-seropositive mothers were infected by HTLV-I. However, the frequency of HTLV-I transmission from mother to cord-blood lymphocytes is 7%.[7] The difference between the frequency of HTLV-I infection of placenta and that of HTLV-I transmission to cord-blood lymphocytes suggests defence mechanisms against HTLV-I infection at the maternofetal interface.

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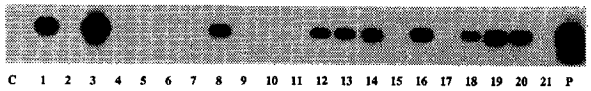
Detection of HTLV-II in breastmilk of HTLV-II infected mothers

SIR,—Human T cell lymphotropic virus type II (HTLV-II) has been reported among American and European intravenous drug users (IVDUs)[1,2] and among American Indian populations, including the Guaymi of Panama.[3] The virus is present in at least half the HTLV-I/II seropositive blood donors in the USA.[4] Since seropositive donors are routinely notified and counselled, accurate information about the modes of transmission and disease associations of HTLV-II is important. Like the closely related



HTLV-II PCR analysis of breastmilk cells.

Samples from 12 HTLV-II-infected (1, 2, 3, 8, 9, 12, 13, 14, 16, 18, 19, 20) and 9 HTLV-II-non-infected individuals (4, 5, 6, 7, 10, 11, 15, 17, 21). Lane P = positive control (Mo-T cells, HTLV-II-infected), lane C = PCR reagents control (no template).

HTLV-I, HTLV-II can be transmitted through contaminated blood products,[5] needle sharing among IVDUs,[4] and by sexual contact.[6] Whereas HTLV-I can be transmitted from mother to child, primarily by breastfeeding,[7] transmission of HTLV-II from mothers to non-breastfed infants is rare.[8] Information about transmission of HTLV-II by breastfeeding is unavailable. Since most HTLV-II-infected blood donors are women,[4] this issue is important when counselling these and other HTLV-II-infected women of childbearing age.

The presence of HTLV-II in the breastmilk of infected mothers constitutes a prerequisite for possible transmission of the virus by breastfeeding. To detect HTLV-II in breastmilk samples of HTLV-II-infected mothers, we used the polymerase chain reaction (PCR) to test lysates of cell pellets of milk samples from 12 HTLV-II-infected and 9 non-infected Guaymi mothers. The infection status of all Guaymi individuals had been determined by serological and/or PCR testing.[3,9] 6–10 ml milk was spun at low speed and the cell pellet was washed twice with phosphate-buffered saline and digested, in PCR buffer containing proteinase K, at 6×10^6 cells per ml.[9] The competence of the DNA template of all preparations was checked by amplification of a sequence from the myeloperoxidase gene. Proviral amplification was done blind on 25 µl lysate with the pol SK110/111 primer pair followed by Southern blot hybridisation to a ^{32}P-labelled SK188 internal probe.[9] 10 of 12 samples (83·3%) from the infected subjects were positive for HTLV-II, whereas all samples from the uninfected mothers were negative (figure).

The presence of HTLV-II-infected cells in the breastmilk of a large proportion of infected subjects raises the possibility of transmission of the virus by breastfeeding. Similar results have been described for HTLV-I,[10] and transmission of HTLV-I by breastfeeding has been documented.[7] We are monitoring the babies born to these Guaymi mothers for evidence of HTLV-II infection.

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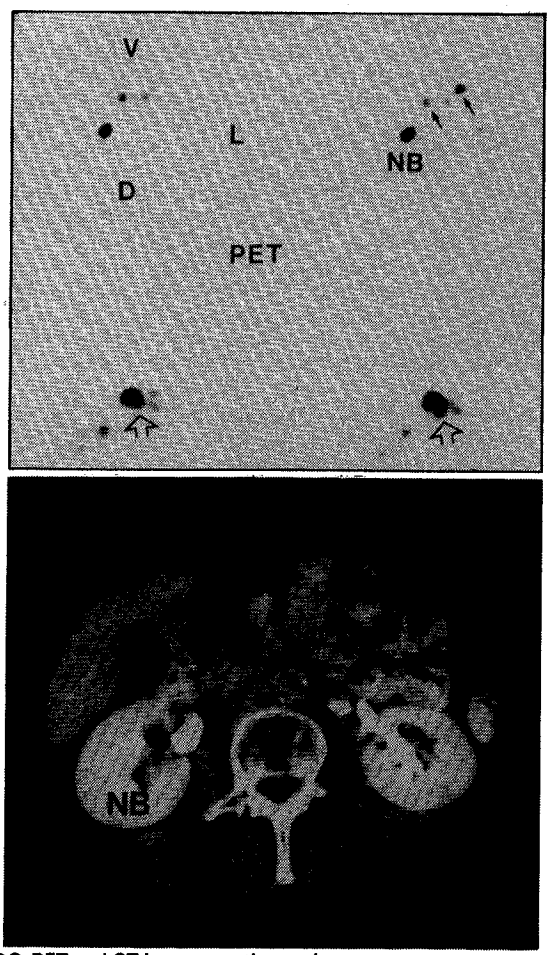
PET with fluorine-18 deoxyglucose for pancreatic disease

SIR,—Positron emission tomography (PET) after injection of ^{18}F-deoxyglucose (FDG) is an alternative approach for detection of pancreatic tumours. The method is based on findings by Warburg,[1] who described increased glucose uptake of malignant tumours compared with normal tissues. We have investigated whether this finding also applied in pancreatic disease, especially whether FDG-PET could discriminate carcinoma from chronic inflammatory processes.

PET (ECAT 953/15, CTI/Siemens) of the upper abdomen was done in 19 patients with suspected carcinoma of the pancreas (13 cases) or history of chronic pancreatitis (6). 45–60 min after injection of 150–300 MBq ^{18}FDG into a peripheral vein, a static PET scan (3 bed positions 15 cm axial field of view) was obtained over 50–70 min. Data for attenuation correction were measured with a ^{68}Ge ring source before FDG injection. Regional FDG uptake was quantified by calculation of standardised uptake values.[2] All patients were injected after an overnight fast. The results with PET were assessed by histopathological examination of the resected carcinoma in 12 patients. In 7 patients diagnosis was established by computed tomography (CT) or magnetic resonance imaging and clinical follow-up for at least 6 months.

FDG uptake in patients with pancreatic carcinoma (n = 11) was significantly higher (mean uptake value 4·3, range 2·3–7·3; tissue concentration in nCi/g ÷ [intravenous activity in nCi ÷ weight in g]) than in those with chronic pancreatitis (n = 8, mean 1·2, range 0·8–1·5; *t* test, $p < 0.01$). The figure compares FDG-PET and CT. The tumour and lymph nodes were not visualised by CT.



FDG-PET and CT in pancreatic carcinoma.

Upper = 4 transaxial FDG-PET scans. Lower = transaxial CT of PET region of interest. V = ventral, D = dorsal, NB = kidney pelvis. Open arrow = carcinoma, closed arrow = lymph nodes.

Unknown metastases of the liver or peripancreatic lymph nodes were demonstrated in 6 patients. In 8 patients benign pancreatic disease was correctly diagnosed by PET. One false-positive was probably due to increased FDG uptake in granulation tissue after previous gastric surgery.

Our study indicates that FDG-PET might improve preoperative diagnosis of pancreatic cancer. Similar findings have been reported by Zanzi et al,[3] who investigated 8 patients with pancreatic carcinoma by FDG-PET. Syrota et al,[4] however, using PET with ^{11}C-labelled L-methionine, could not differentiate carcinoma from inflammatory pancreatic processes. We feel that FDG-PET is a promising tool for diagnosis of pancreatic cancer. By making early diagnosis more feasible, the technique may even help to improve prognosis.

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Urinary 5HTOL/5HIAA as biochemical marker of postmortem ethanol synthesis

SIR,—The presence of ethanol in body fluids is important when accidents are investigated. However, postmortem synthesis is a constant dilemma.[1] Because ethanol can be produced from glucose and other substrates between death and necropsy, addition of fluoride or other preservative to body fluids submitted for analysis does not rule out artifactual ethanol production.

Ethanol can alter the catabolism of serotonin (5-hydroxytryptamine).[2] Normally, most serotonin is oxidised to 5-hydroxyindoleacetic acid (5HIAA) while some is reduced to 5-hydroxytryptophol (5HTOL). Both metabolites are excreted in urine and the molar ratio 5HTOL/5HIAA is usually below 0·01. However, after drinking alcohol, the 5HTOL/5HIAA ratio is increased.[2,3] Because 5HTOL and 5HIAA are stable on storage,[4,5] we investigated whether examination of 5HTOL/5HIAA in urine specimens obtained postmortem might be a way to distinguish antemortem ingestion of ethanol from postmortem synthesis.

We measured the concentration of ethanol by head-space gas chromatography[6] in 22 blood and urine samples selected at random during forensic necropsies. Urinary 5HTOL and 5HIAA were assayed by gas-chromatography/mass-spectrometry and high-performance liquid chromatography, respectively.[4,5] Correlation between urinary ethanol and 5HTOL/5HIAA ratio was good ($r = 0·73$, $p < 0·001$), and in all urine specimens with detectable ethanol, 5HTOL/5HIAA was increased. In 1 case, postmortem ethanol formation was suspected, because blood alcohol concentration was 16·8 mmol/l (80 mg/dl) and urinary ethanol was zero. The 5HTOL/5HIAA ratio in urine was normal, which confirmed our suspicion of postmortem ethanol synthesis in the blood.

Further support for the use of this method in forensic toxicology was obtained from an experiment with fresh urine samples spiked with glucose 2% and *Candida albicans* 1000 colony-forming units per ml. This resulted in the formation of large amounts of ethanol, but 5HTOL/5HIAA ratio remained unchanged:

Days	*Ethanol (mmol/l)*		*5HTOL/5HIAA ($\times 10^3$)*	
(room temperature)	Urine 1	Urine 2	Urine 1	Urine 2
0	0	0	3·8	2·0
1	0	0	3·7	1·8
4	65	9	3·9	1·9
7	171	71	3·6	1·8

These preliminary results are encouraging and the 5HTOL/5HIAA ratio in urine provides a useful complement to other potential markers of postmortem ethanol synthesis, such as identification of higher alcohols (*n*-propanol and *n*-butanol) or comparing ethanol levels in urine and vitreous humour with those in blood from the heart and peripheral veins.[1] We are investigating whether the 5HTOL/5HIAA ratio can also be used with forensic blood samples.

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Travellers' diarrhoea associated with cyanobacterium-like bodies

SIR,—Dr Pollok and colleagues (Aug 29, p 556) report that cyanobacterium-like bodies were detected in faeces from patients with diarrhoea. Microcystines and nodularin, the toxic substances produced by fresh water cyanobacteria (or blue-green algae), have been identified as potent and selective inhibitors of protein phosphatases.[1,2] Correlation between diarrhoeal illness and phosphatase inhibitors has been shown in shellfish poisoning. Poisoning by European mussel and Japanese mussel is due to okadaic acid and 35-methyl okadaic acid, respectively,[3] and both these toxins are potent and selective phosphatase inhibitors.[4,5]

Protein phosphorylation and dephosphorylation regulate cell functions. Phosphatase inhibitors change the balance between the activities of protein kinase and phosphatase and modify various cell functions such as the motility of gastrointestinal smooth muscle and release of neurotransmitters.[6,7] These effects may result in diarrhoeal illness. In addition okadaic acid might cause diarrhoea by increasing the phosphorylation of the same protein as that phosphorylated by a toxin secreted from *Vibrio cholerae* and which increases the sodium and potassium secretion by intestinal cells.[4] To understand the role of phosphatase inhibition in this pathogenesis, the protein whose phosphorylation is increased by these phosphatase inhibitors will need to be identified.

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